The impact of *CACNA1C* gene, and its epistasis with *ZNF804A*, on white matter microstructure in health, schizophrenia and bipolar disorder

Short title: CACNA1C impacts on white matter microstructure

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Keywords: *genome-wide association, diffusion tensor imaging, fractional anisotropy, white matter, schizophrenia, bipolar disorder, CACNA1C, rs1006737, ZNF804A epistasis*

Date of Submission: 30th June, 2016

Word Counts

Abstract: 239

Introduction: 801

Discussion: 1614

Abbreviations: *ANOVA, analysis of variance; BD, bipolar disorder; CAC+/-, psychosis risk/protective genotype for CACNA1C rs1006737; CACNA1C, Calcium Voltage-Gated Channel Subunit Alpha1 C; CNS, central nervous system; CPZ, chlorpromazine; DTI, diffusion tensor imaging; FA, fractional anisotropy; FSL, Functional MRI of the Brain lab software library; GWAS, genome-wide association study; HWE, Hardy-Weinberg Equilibrium; SNP, single nucleotide polymorphism; TBSS, tract-based spatial statistics; TFCE, threshold-free cluster enhancement; WM, white matter; ZNF+/-, psychosis risk/protective genotype for ZNF804A rs1344706; ZNF804A, Zinc Finger Protein 804A; SZ, schizophrenia.*

Abstract

Genome-wide studies have identified allele A (adenine) of single nucleotide polymorphism (SNP) rs1006737 of the calcium-channel *CACNA1C* gene as a risk factor for both schizophrenia (SZ) and bipolar disorder (BD) as well as allele A for rs1344706 in the zinc-finger *ZNF804A* gene. These illnesses have also been associated with white matter abnormalities, reflected by reductions in fractional anisotropy (FA), measured using diffusion tensor imaging (DTI). We assessed the impact of the *CACNA1C* psychosis risk variant on FA in SZ, BD and health. 230 individuals (with existing *ZNF804A* rs1344706 genotype data) were genotyped for *CACNA1C* rs1006737 and underwent DTI. FA data was analysed with tract-based spatial statistics and threshold-free cluster enhancement significance correction ($p<0.05$) to detect effects of *CACNA1C* genotype on FA, and its potential interaction with *ZNF804A* genotype and with diagnosis, on FA. There was no significant main effect of the *CACNA1C* genotype on FA, nor diagnosis by genotype(s) interactions. Nevertheless, when inspecting SZ in particular, risk allele carriers had significantly lower FA than the protective genotype individuals, in portions of the left middle occipital and parahippocampal gyri, right cerebelleum, left optic radiation and left inferior and superior temporal gyri. Our data suggests a minor involvement of *CACNA1C* rs1006737 in psychosis via conferring susceptibility to white matter microstructural abnormalities in SZ. Put in perspective, *ZNF804A* rs1344706, not only had a significant main effect, but its SZ-specific effects were two orders of magnitude more widespread than that of *CACNA1C* rs1006737.

Introduction

Schizophrenia (SZ) and bipolar disorder (BD) are major psychiatric illnesses that have profound effects on a person's mood, cognition and behavior. Psychotic and negative symptomatology, and cognitive impairments are common to both, albeit these are more severe for SZ (Green *et al.*, 2010). Lifetime prevalence of SZ and BD is about 4% (Bhugra, 2005) and 0.5% respectively (Merikangas *et al.*, 2007). Both illnesses are highly heritable: up to 80% (SZ) and 93% (BD) (Gurung & Prata, 2015) but their common and specific aetiological and pathophysiological causes are poorly understood.

Genome-wide association studies (GWAS) have identified two genetic variants conferring, so far the most reproducible, risk for both SZ and BD: allele A of the single nucleotide polymorphism (SNP) labelled rs1006737 of the CACNA1C gene (Ferreira *et al.*, 2008, Green *et al.*, 2010, Guan *et al.*, 2014, He *et al.*, 2014, Jiang *et al.*, 2015, Nyegaard *et al.*, 2010, Sklar *et al.*, 2008, Zheng *et al.*, 2014) and allele A of the rs1344706 SNP of the ZNF804A gene (Donohoe *et al.*, 2010, Sun *et al.*, 2015, Zhang *et al.*, 2015, Zhu *et al.*, 2014). The latter variant has been associated with white matter (WM) microstructural abnormalities as we report elsewhere (Mallas *et al.*, 2016).

The CACNA1C gene, located on the short arm of chromosome 12p13.3 encodes an alpha-1 subunit of a voltage dependent L-type calcium-channel (LTCC) referred to as $Ca_V1.2$. LTCCs regulate the influx of calcium ions into the cell upon polarisation of the membrane, and, being widely expressed in the central nervous system (CNS), are

involved in various processes including regulation of signalling pathways, neurotransmitter release and neuronal excitability. $Ca_V1.2$ is thought to be important in synaptic plasticity and neuronal gene expression (Uemura *et al.*, 2015).

SZ and BD have also been robustly associated with WM abnormalities. Fractional anisotropy (FA) measured using diffusion tensor imaging (DTI) is a putative proxy of WM microstructural integrity (Jones *et al.*, 2013) and decreases in FA can thus be regarded as being indicative of reduced WM integrity. Additionally, FA is highly heritable: 75-90% of the variance in FA across almost all WM regions could be explained by genetic factors (Chiang *et al.*, 2009). Several studies have reported FA reductions in psychotic probands, with similar abnormalities to an attenuated degree, observed in healthy first-degree relatives (Chaddock *et al.*, 2009, Skudlarski *et al.*, 2013, Sprooten *et al.*, 2012), and FA has been found to decrease with increasing genetic liability to psychosis (Emsell *et al.*, 2013, Phillips *et al.*, 2011). Reduced FA is a replicated finding in SZ, and to a lesser extent, in BD, in a diverse range of brain regions (Ellison-Wright & Bullmore, 2009, Vederine *et al.*, 2011). Furthermore, reduced FA can be detected in both the prodromal and early stages of illness (Carletti *et al.*, 2012, Cheung *et al.*, 2008, Karlsgodt *et al.*, 2008, Szeszko *et al.*, 2005), suggesting that microstructural abnormalities in WM are involved in the underlying neuropathophysiology of the disease.

Imaging genetics evidence of the impact of *CACNA1C* rs1006737 on WM microstructure is sparse. The risk allele has recently been associated with reduced FA in the right hippocampal formation in Caucasian healthy subjects in a study

researching this location only (Dietsche *et al.*, 2014). In another study whole-brain but non WM-tract-based, SZ Chinese patients carrying the risk allele have also shown lower mean FA in the left frontal lobe, left parietal lobe and left temporal lobe, compared to G homozygotes while healthy G homozygotes show higher mean FA compared to A-allele carriers in the right cingulate gyrus and left temporal lobe (Woon *et al.*, 2014). However, none of these studies have provided whole-brain WM tract-based evidence of an impact on FA, thus providing limited resolution of this SNP's effects. Moreover, no study has researched the impact of this SNP in a clinical population.

In this study we aimed to assess for the first time the effect of the genome-wide risk variant allele A for rs1006737 of *CACNA1C* on tract-based regional FA throughout the whole brain in a large (n=230) and predominantly Caucasian (83%) sample of non-clinical controls and SZ and BD patients. Unprecedentedly, we also enquire if *CACNA1C* rs1006737 is modulated by diagnosis and by the other most GWAS-implicated SNP: the rs1344706 of the ZNF804A gene (for our review of GWAS-implicated genes on brain phenotypes, see (Gurung & Prata, 2015); the impact of the ZNF804A gene on psychiatric disorders is discussed in more detail in our previously reported work (Mallas *et al.*, 2016). We predicted that the risk allele (A) of *CACNA1C* rs1006737 would be associated with reduced FA across all subjects, irrespective of diagnosis, but might have a greater effect in the patient (SZ and BD) groups. We also anticipated that the effect of this risk allele might be augmented by the presence of the risk allele A of *ZNF804A* rs1344706.

Material and Methods

Participants

The total sample of 230 subjects consisted of patients with SZ (n=63), patients with BD (type 1 or type 2; 77% of which with psychosis; n=43) and non-clinical controls (n=124), who had participated in seven previous research imaging studies at the Institute of Psychiatry, Psychology & Neuroscience, King's College London (Allin *et al.*, 2011, Chaddock, 2009, Chaddock *et al.*, 2009, Kyriakopoulos *et al.*, 2009, Picchioni *et al.*, 2006, Shergill *et al.*, 2007). Individuals were collated from those sub-samples, with any relatives excluded, as detailed elsewhere (Mallas *et al.*, 2016).

Each participant was assigned to three groups: a diagnosis group (SZ, BD or control), and after genotyping (see below), a genotype group for *CACNA1C* in terms of risk allele load (CAC+: A homozygotes and heterozygotes; and CAC-: allele G (guanine) homozygotes), and for *ZNF804A* (ZNF+: A homozygotes; and ZNF-: heterozygotes and C (cytosine) homozygotes). ZNF grouping is discussed in Mallas *et al.* (2016). Cell sizes (averaging 19.2 subjects) are shown in Supplementary Material 1. The grouping of genotype groups had the purpose of maximising counterbalance for the rs1006737 *CACNA1C* SNP, as commonly practiced in the literature (Backes *et al.*, 2014, Dietsche *et al.*, 2014, Nieratschker *et al.*, 2015, Ou *et al.*, 2015, Woon *et al.*, 2014) given the much lower frequency counts of allele A, compared to C, in the Caucasian population. The SNPs reported in this manuscript are the only SNPs analysed in this imaging data set.

The study was approved by the National Health Service (NHS) South East London Research Ethics Committee, UK (Project "Genetics and Psychosis (GAP)" reference number 047/04). All subjects provided written informed consent at the time of participation. Patients were recruited from the South London and Maudsley (SLaM) NHS Trust. Diagnosis, according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM) 4th Edition (American.Psychiatric.Association & Dsm-Iv., 1994) was ascertained by an experienced psychiatrist using a structured diagnostic interview with instruments detailed elsewhere (Prata *et al.*, 2009). All SZ and BD patients were in a stable clinical state and all SZ and some BD were treated with antipsychotic medication (from which Chlorpromazine-equivalence was calculated, see Table 1).

Exclusion criteria applied to all participants were a history of significant head injury and current (last 12 months) substance dependency according to DSM-IV diagnostic criteria. Non-clinical controls were excluded if they had any personal or family history of a psychotic spectrum disorder. In order to follow the gold standard of experimental design that a control group must be matched to the experimental group on all variables except the one isolated for study, and avoid a biased 'super-normal' control group (Kendler, 2003), participants with a previous, but not present, diagnosis of any other Axis I disorder (or family history) were not excluded given these are frequently present in SZ and BD (as such, two controls had once in the past been diagnosed with mild depression). None were psychiatrically unwell or on any psychiatric medication at the time of participation.

Genotyping

DNA was extracted from blood samples or buccal swabs following a standard protocol (Freeman *et al.*, 1997, Freeman *et al.*, 2003) and a TaqMan SNP Genotyping Assay (Applied.Biosystems, 2010) for *CACNA1C* SNP rs1006737 (A/G) and *ZNF804A* rs1344706 (A/C) was performed blind to any phenotype data, at the Social Genetic and Developmental Psychiatry Centre (SGDP) lab, King's College London.

Genotype counts did not differ significantly from those quoted in the National Center for Biotechnology Information (NCBI) database for *CACNA1C* rs1006737 (92 GG, 96 AG, 42 AA, χ^2=0.41, df=2, p=0.815) nor deviated significantly ($p<0.05$) from Hardy-Weinberg Equilibrium (HWE) in Caucasians (χ^2= 1.33, p=0.25, df=1); HWE could not be calculated for the other ethnicities due to insufficient counts for a χ^2 test, in each. HWE was calculated using Michael H. Court's online calculator (Court, 2005-2008). There were no further polymorphisms genotyped in this sample.

Image Acquisition

Magnetic Resonance Imaging (MRI) data were acquired using a 1.5T GE Signal LX system (General Electric, Milwaukee, WI, USA) in the Mapother House MR unit at the Maudsley Hospital, South London and Maudsley (SLaM) NHS Foundation Trust, London, UK. The scanner had actively shielded magnetic field gradients (maximum amplitude 40mT/m1), and a quadrature birdcage head coil was used for both radiofrequency (RF) transmission and signal reception. Each volume of DTI data was

acquired using a multi-slice peripherally-gated echo planar imaging (EPI) sequence, optimised for precise measurement of the diffusion tensor in parenchyma, from 60 contiguous near-axial slice locations for whole brain coverage, with isotropic (2.5x2.5x2.5 mm) voxel size. At each slice location, 7 images were acquired with no diffusion gradients applied (b=0), together with 64 diffusion-weighted images in which gradient directions were uniformly distributed in space. Acquisition parameters were as follows: echo time (TE) =107ms, effective repetition time =15 R-R intervals, duration of the diffusion encoding gradients = 17.3ms, with a maximum diffusion weighting =1300s/mm^2. Further details of the methodology are given elsewhere (Jones *et al.*, 2002).

DTI Data Processing

The raw DTI data were corrected for head movement and eddy-current induced distortions, and then brain-extracted using the Brain Extraction Tool (BET) (Smith, 2002) in order to exclude non-brain voxels. After visual inspection of images, the BET threshold was adjusted to 0.2 to ensure a balance between complete scalp removal and inappropriate erosion of brain tissue that had not been achieved using the default parameter setting of 0.5. FA images were then created (with a mask defined by a binarised version of this brain-extracted image) by fitting a tensor model to the raw diffusion data using the Functional MRI of the Brain lab (FMRIB)'s Diffusion Toolbox (FDT) within FMRIB software library (FSL) as described elsewhere (Behrens *et al.*, 2003).

Voxel-wise statistical analysis of the FA data was carried out using tract-based spatial statistics (TBSS) (Smith *et al.*, 2006), also part of FSL (Smith *et al.*, 2004). Briefly: all subjects' FA data were aligned to FMRIB58_FA 1x1x1mm standard space (an average of the FA images of 58 healthy adults) using the nonlinear registration tool FNIRT (Andersson *et al.*, 2007a, Andersson *et al.*, 2007b), which uses a b-spline representation of the registration warp field (Rueckert *et al.*, 1999). The entire aligned dataset was then affine-transformed into a 1x1x1mm MNI152 space, resulting in a standard space version of each subject's FA image, from which the mean FA image was created and thinned to create a mean FA skeleton. Each subject's aligned FA data were then projected onto this skeleton and the resulting data fed into voxel-wise cross-subject statistics.

Group-level Statistical Analyses

Demographic differences between diagnostic or genotype groups, were tested using independent t-tests, Chi-Square and Analysis of Variance (ANOVA) in Statistical Package for Social Sciences (SPSS) v21 (Corp., 2012) .

The FSL Randomise tool (Anderson & Robinson, 2001) was used to perform permutation-based non-parametric inferences on the skeletonised FA data at a threshold of 0.2 (TBSS default) with 10,000 permutations. The significance level was set at $p<0.05$ after multiple comparisons correction using threshold-free cluster enhancement (TFCE) (Smith & Nichols, 2009), an approach that allows the significance of a target voxel to take into account not only the amplitude of the signal (in this case FA) but also the contribution of both the spatial extent and the magnitude

of supporting voxels. To assess the main effect of *CACNA1C* genotype, of *ZNF804A* genotype, and of diagnostic group as well as their interactions on FA, an ANOVA design matrix was built with *CACNA1C* genotype (CAC+ vs. CAC-), *ZNF804A* genotype (ZNF+ vs. ZNF-) and diagnosis (SZ, BD and controls) as the three independent variables, and age as a covariate in effects where age differed significantly across groups. The main effect of diagnosis and of ZNF has been reported in detail elsewhere (Mallas *et al.*, 2016). We will also report simple effects for completeness. The 'Cluster' tool in FSL was used to define clusters within the statistical images. The mean FA within the largest cluster of each effect was graphically plotted for visual display of effects.

WM labelling based on the JHU ICBM-DTI-81 WM Atlas (Mori *et al.*, 2008) provided by the Atlasquery tool FSL, was used to determine the anatomical location of significant FA clusters; only with greater than 1% probability of being part of the WM in question were included in the cluster table. Where cluster results were retrieved as 'Unclassified', labelling was carried out manually by FC using the MRI Atlas of Human WM (Mori *et al.*, 2005). Results were overlaid on MNI152 (1mm) standard template and displayed in radiological convention.

Results

Demographics

Participant demographic data are shown in Table 1. BD patients (mean age = 41.1, SD =12.3) were significantly older than SZ patients (mean age =33.8, SD =10.7) and controls (mean age = 35.8, SD =13.40), while there was no significant difference in age between the control and the SZ group. There were significantly (χ^2 =17.2, p<0.001) more males (50M:13F) in SZ than BD (18M:25F) or control (67M:57F) groups. There was a significant difference (χ^2 =11.17, df =4, p =0.03) in genotype distribution between diagnostic groups. SZ patients (mean CPZ score =696.9, SD =613.0) had a significantly higher (t(104) =3.3, p<0.001) CPZ-equivalent score than BD (mean CPZ score = 341.6, SD =434.6). There were no other significant differences between diagnostic groups in IQ, years of education, ethnicity or handedness. There was a significant difference in the distribution of ethnicities between CAC+ and CAC- (χ^2 = 14.37, df =6, p =0.03) but no other significant differences between the genotype groups in IQ, age, chlorpromazine (CPZ) equivalents in medication, years of education or handedness. *ZNF804A* genotype-wise demographics differed only for ethnicity (χ^2 =20.86, df =6, p<0.001) given the a priori different allele frequencies between African and Caucasian populations. Full details are reported elsewhere (Mallas *et al.*, 2016) and displayed in Supporting Material 2.

Effect of *CACNA1C* Genotype on FA

Whole-brain, voxel-based TBSS retrieved no significant main effect of *CACNA1C* rs1006737 genotype on FA in the total sample. However, as a secondary result, there was a significant (p<0.05, TFCE-corrected) simple effect of genotype within the SZ

group alone (Figure 1a), in which FA was significantly lower in the SZ CAC+ than in the SZ CAC- group. This effect encompasses WM portions of the left middle occipital and parahippocampal gyri, right cerebelleum, left optic radiation and left inferior and superior temporal gyri (Figure 1c; Table 2). No effect in the opposite direction was significant in any location, either as a main effect or within SZ, BD or controls separately.

Interactions involving *CACNA1C* Genotype, ZNF804A Genotype and/or diagnosis on FA

Before correcting for age, there was a significant ($p<0.05$, TFCE-corrected) 3-way interaction between *CACNA1C* rs1006737 genotype, *ZNF804A* rs1344706 genotype and diagnosis affecting the body of the corpus callosum, the right superior corona radiata and the left anterior corona radiata, the largest cluster spanning 125 voxels. This reflected that the presence of the risk genotype of *ZNF804A* (ZNF+; vs. ZNF-) increased the magnitude of the effect of the *CACNA1C* risk genotype (CAC+; vs. CAC-) on FA, in BD patients more than in controls. However, this effect was no longer significant when age (which was significantly higher in BD than controls or SZ groups) was added into the model as a covariate. No other locations showed alternative interaction patterns of this 3-way ANOVA.

Effect of Diagnosis on FA

Both SZ and BD had significantly reduced FA compared to the control group. There was no significant difference in FA between patient groups, nor were there regions

where FA was significantly decreased in the control group compared to patient groups. We have previously reported this in detail (Mallas *et al.*, 2016).

Discussion

We assessed the effect across the human brain of the *CACNA1C* rs1006737 genotype on FA, unprecedentedly in a Caucasian clinical sample as well as in health, and whether this genotype effect was different between diagnostic groups and whether it interacted with the *ZNF804A* rs1344706 genotype. Although using a relatively large sample for imaging analysis, we detected no significant ($p<0.05$, TFCE-corrected) main effects or interaction effects from an ANOVA.

As a result of a complementary analysis to our main hypotheses of main and interaction effects, we detected statistically significant, albeit small, effect ($p<0.05$, TFCE-corrected), in the predicted direction, in our SZ sample alone. This is the first evidence of an association between *CACNA1C* rs1006737 risk allele A and reduced FA in a predominantly Caucasian clinical sample. This result may suggest the involvement of the GWA-implicated linkage disequilibrium region tagged by allele A at *CACNA1C* rs1006737 locus in influencing susceptibility to psychosis by demonstrating its effect in reducing FA in the SZ population. This putatively reflects changes in WM microstructure and reduction of WM integrity in SZ. Comparatively, both the effect in SZ and the overall main effect of genotype that we found for another top GWA-implicated *ZNF804A* rs1344706 was much larger and more spatially expansive (Mallas *et al.*, 2016): the main effect of *ZNF804A* genotype spanned more than 44,054 voxels in the largest cluster and its effect in SZ only, spanned 51,260 voxels. Thus, one should put these polymorphisms' effect in perspective: both the effects of *ZNF804A* genotype spanned an location of about 2 orders of magnitude larger than the one under the CACNA1C genotype effects we report herein.

Given that we found an effect of *CACNA1C* in SZ but not in controls, but a *CACNA1C* genotype by diagnosis interaction was not significant, it is possible that power was not sufficient to detect the latter. We encourage further studies to use a larger sample study to detect whether SZ does indeed increase susceptibility to a *CACNA1C* rs1006737 effects. Our finding that FA in SZ patients carrying the risk allele was significantly lower than in SZ non-risk allele carriers is consistent with those reported by Woon *et al.* (2014) in a Chinese SZ population of similar size (96 SZ and 64 controls). However, in our study we found an effect in the left middle occipital gyrus, left parahippocampal gyrus, left optic radiation and left temporal gyrus WM, while Woon et al. (2014) reported the left frontal lobe, left parietal lobe and left temporal lobe WM. We found significantly reduced FA in the right cerebellar WM, whilst Woon et al. (2014) found it on the left. Unfortunately, precise comparisons with their study are limited by the different approaches: while we report comparatively more precise (TBSS) tract-based or gyri, they report very wide lobes.

In our non-clinical volunteers, the high risk A allele carriers did not differ significantly in any brain region's FA compared to the low risk G homozygotes, for *CACNA1C* rs1006737. This is inconsistent with recent positive findings reported by Dietsche *et al.* (2014) of decreased FA in the right hippocampal formation in non-clinical A allele carriers. A possible explanation is that Dietsche et al. (2014) focused on one region of interest (ROI) in contrast to our whole-brain analysis, thus allowing a more relaxed multiple comparisons correction in their selected location.

We also report the first test of a *CACNA1C* and *ZNF804A* interaction. We predicted their respective GWAs-implicated risk SNPs would interact in an additive manner, the risk

increasing when both risk allele groups were present (e.g. CAC+ and ZNF+) – in consistency with the hypothesis that both these polymorphisms increase the risk for psychosis, and that psychosis is associated with a (widespread) decrease in FA. Prior to covarying for age, we found, albeit only in BD compared to controls (and surprisingly not in SZ vs. controls), that the concomitant presence of both risk alleles was represented more in subjects with relatively decreased FA in the body of the corpus callosum, the right superior corona radiata and the left anterior corona radiata. However, when we added age as a covariate to the model this effect did not survive significance, suggesting that the difference in BD patients' age (vs controls or SZ) across genotype groups had driven this interaction effect. Further research therefore needs to explore this effect in a larger, age matched sample.

Structural volumetric imaging studies have also implicated the *CACNA1C* rs1006737 risk-allele in increased regional (Frazier *et al.*, 2014) and total (Kempton *et al.*, 2009, Wang *et al.*, 2011) gray matter volumes in non-clinical controls, inclusive in a dose-dependent manner (Kempton *et al.*, 2009). Others have found the same trend in the amygdala and hypothalamus (Perrier *et al.*, 2011) irrespective of diagnosis, and, in SZ patients specifically, the amygdala (Wolf *et al.*, 2014). However, a larger sample of almost 600 healthy subjects failed to replicate this association (Franke *et al.*, 2010), as did another study in amygdala and hippocampal volumes, in BD patients and healthy controls (Soeiro-De-Souza *et al.*, 2012). Regarding WM, the risk allele has also been associated with increased total and fronto-limbic volumes (Frazier *et al.*, 2014).

Functional MRI studies have also implicated the *CACNA1C* rs1006737 risk allele (A) in candidate behavioural intermediate phenotypes for BD and SZ, it being associated with

increased activation (and thus putatively 'inefficient' when performance differences are controlled for) in the left inferior frontal gyrus and left precuneus during a verbal fluency task (Krug *et al.*, 2010), in which performance is often impaired in SZ and BD (Krabbendam *et al.*, 2005). The same allele has also been associated with increased activation in the left amygdala in BD patients in a face-matching task (Tesli *et al.*, 2013), increased hippocampal and prefrontal activation during emotional processing and executive cognition tasks respectively (Bigos *et al.*, 2010), and enhanced right amygdala response both during a monetary reward task (Wessa *et al.*, 2010) and a face recognition task (Jogia *et al.*, 2011). In contrast, response reductions in bilateral hippocampal (Erk *et al.*, 2010), medial frontal gyrus (Thimm *et al.*, 2011) and right dorso-lateral prefrontal cortex (dlPFC) (Paulus *et al.*, 2013) have also been associated with this allele during episodic memory, executive control of attention and working memory tasks respectively. While the findings of reduced activation are harder to reconcile, those of (putatively inefficiently) increased activation during cognitive executive tasks provide converging support to our finding that the same risk allele is influencing WM microstructural abnormalities. Nevertheless, further research combining fMRI-based and DTI-based connectivity would be paramount to adequately test to what extent localized functional cognitive deficits are caused by FA deficits affecting the same regions.

Regarding the influence of the illnesses on the *CACNA1C* rs1006737 genotype effect, Jogia *et al.* (2011) observed a significant genotype x diagnosis interaction in the right ventro-lateral prefrontal cortex, where risk-allele carriers in the BD group, but not unaffected relatives' nor unrelated controls, showed reduced activation. Furthermore, in a partial superset of the current sample, including 20 BD patients, 20 unaffected first-degree relatives and 20 unrelated healthy controls, Radua *et al.* (2013) found a similar genotype x diagnosis interaction, this time on effective brain connectivity. The risk allele A was associated with decreased outflow of

information from the medial frontal gyrus during perception of fearful faces, significantly more so in BD than relatives or controls. This disruption in connectivity is a plausible consequence of WM microstructural abnormalities we found herein and thus supports the idea that this genotypic variation exerts its functional effect, to a greater extreme in an illness context, via a disruption in WM microstructure.

Supporting the plausibility that genetic factors, such as *CACNA1C* rs1006737 A allele, could increase risk of psychosis through predisposing individuals to abnormalities in WM microstructure, are heritability studies of WM. These have suggested that genetic factors explain a large proportion of the variance in WM inter-individual differences (originally estimated at around 88% (Baaré *et al.*, 2001)) with a meta-analysis of over 50 studies further supporting the genetic basis of total and regional WM volumes (Blokland *et al.*, 2012). Mechanism-wise, it is possible that *CACNA1C* rs1006737 exerts its effect through altering levels of *CACNA1C* mRNA expression. As rs1006737 is located in a non-coding region of the gene, it is not thought that this SNP directly interferes with CaV1.2 properties. The risk allele (A) was associated with increased mRNA expression of *CACNA1C* in induced human neurons, and increased density of Cav1.2-mediated currents (Yoshimizu *et al.*, 2014), albeit it was also found to be associated with decreased expression in the human cerebellum (Gershon *et al.*, 2014). As the latter authors state, this may suggest that either an increase or decrease of calcium influx in excitable cells might be associated with BD, as both could lead to changes in monoamine neurotransmitter synthesis and release (Gershon *et al.*, 2014) which has been associated with psychiatric disorders (Booij *et al.*, 2003). Nevertheless, a more comprehensive characterization of this polymorphism's impact on mRNA levels across different brain regions is still warranted.

In conclusion, and in combination with our previous work (Mallas *et al.*, 2016), we provide evidence that the genomic regions tagged by the two most significantly genome-wide associated psychosis risk variants for SZ and BD (in the genes for *CACNA1C* and *ZNF804A*) may increase susceptibility to abnormal WM microstructure and, as such, to psychosis. Effects of the respective causative *ZNF804A* polymorphism were demonstrated to be two orders of magnitude more widespread (and present as a main effect as well as a SZ-specific effect) compared to those of the *CACNA1C* one (where only a SZ-specific effect was found).

Tables and Figures

Figure 1. Effect of *CACNA1C* rs1006737 genotype on fractional anisotropy.

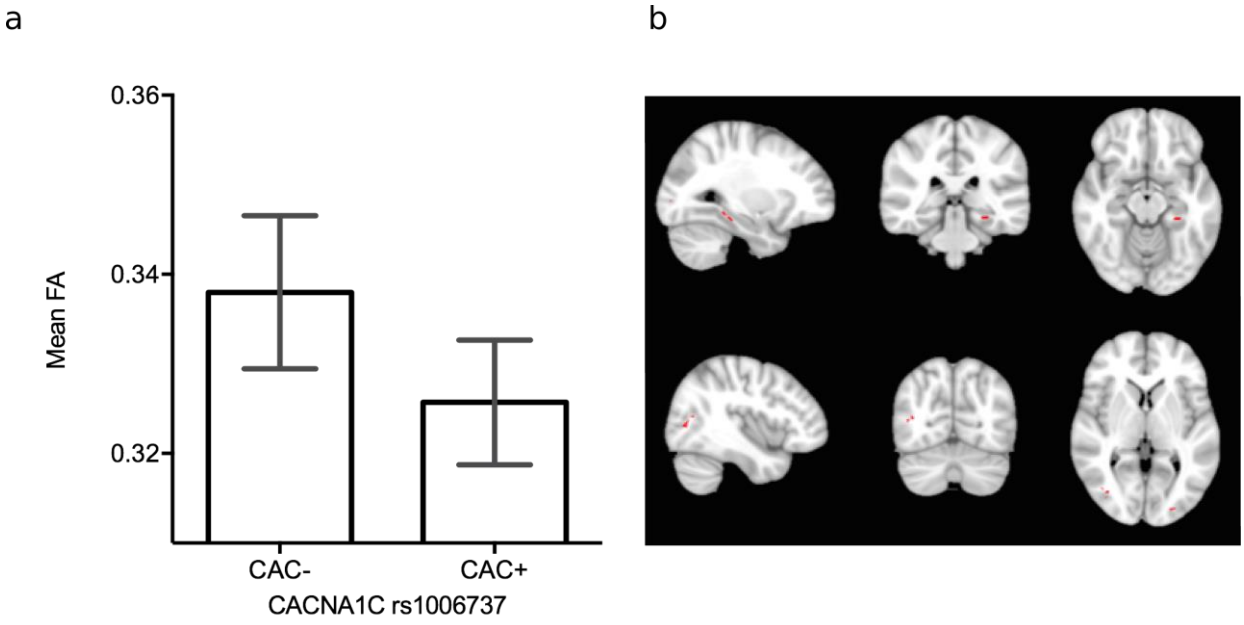

Figure Legend: *Figure 1. a. FA was significantly reduced in SZ CAC+ group (i.e. risk A allele carriers for* rs1006737*) compared to the SZ CAC- group (i.e. protective G allele homozygotes ($p<0.05$, TFCE corrected) in locations labelled in red in this figure's section; b. The difference in FA between CAC+ and CAC- was not statistically significant anywhere in the BD or control groups alone, nor irrespective of diagnosis. b. Figure depicting locations where in SZ, the CAC+ group showed significantly reduced FA compared to the CAC- group. Significant clusters displayed in red overlaid on MNI template in radiological view.*

Table 1. Participant Demographics.

Participant Demographics (n=230)								
		Diagnosis				*CACNA1C* rs1006737 Genotype		
		SZ (n=63)	**BD (n=43)**	**Control (n=124)**	**Test Statistic, df, p-value**	**CAC+ (AA/AG;n=138)**	**CAC- (GG; n=92)**	**Test Statistic, df, p-value**
Age (SD)		33.78 (10.70)	41.07 (12.33)	35.79 (13.40)	F=4.5, df=2, p=0.01†	34.86 (12.56)	38.28 (12.56)	t (228) = 2.018, p=0.05*
IQ: z-scores (SD)§		-0.75 (2.89)	-0.87 (0.97)	-0.68 (3.51)	F=0.70, df=2, p=0.50	-0.60 (2.78)	-0.56 (3.27)	t (197) = 0.08, p=0.94
Antipsychotics dose (in CPZ equivalent; SD)		696.94 (613.02)	341.60 (434.56)	n/a	F=10.75, df=1, p=0.00†	291.15 (530.70)	200.18 (376.18)	t (227.11) = -1.52, p=0.13
Years of Education (SD)		13.74 (2.61)	14.81 (3.10)	14.90 (2.79)	F=2.51, df=2, p=0.08	14.57 (2.98)	14.57 (2.68)	t (162) = 0.01, p =0.99
Sex (M/F)		50/13	18/25	67/57	χ^2=17.24, df=2, p=0.00†	83/55	52/40	χ^2 = 0.30, df=1, p=0.59
Ethnicity	**White**	46	40	104	χ^2=13.90, df=12, p=0.31	112	78	χ^2=14.37, df=6, p=0.03*
	Black Caribbean	6	1	4		10	1	
	Black African	5	2	6		9	4	
	Central Asian	3	0	4		5	2	
	Mixed African and White	2	0	1		2	1	
	Eastern Asian	0	0	3		0	3	
	Other	1	0	2		0	3	
Handedness	**Right**	62	38	112	χ^2=5.79, df=4, p=0.22	128	84	χ^2=0.35, df=2, p=0.84
	Left	0	3	5		4	4	
	Mixed	1	2	7		6	4	
Genotype (%)	**AA**	10 (16%)	15 (35%)	17 (14%)	χ^2=11.17, df=4, p=0.03*			
	AG	30 (48%)	15 (35%)	51 (41%)				
	GG	23 (37%)	13 (30%)	56 (45%)				

Footnote: n/a = not applicable; †statistically significant at 95% level. CAC+: High risk (AA/AG genotypes); CAC-: Low risk (GG genotypes); BD: Bipolar Disorder; SZ: Schizophrenia; SD: Standard deviation; df: degrees of freedom.

§Scores of full scale IQ from the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999), the Wechsler Adult Intelligence Scale – Revised (WAIS-R) (Wechsler, 1981) or the National Adult Reading Test (NART) (Nelson & Willison, 1991), were standardised to Z-scores to permit between-group demographic comparison. The type of test used was balanced between diagnostic or genotype groups.

Table 2: White matter labels corresponding to clusters showing significant effects on fractional anisotropy (p<0.05 after TFCE correction).

Cluster Size (Voxels)	Cluster Maximum Z-statistic	Cluster MAX Co-ordinates X	Y	Z	White Matter Labels [a]
		SZ-specific effect *CACNA1C* (CAC+<CAC-)			
133	0.982	44	-69	8	WM of the L middle occipital gyrus
60	0.975	-31	-31	-15	WM of the L parahippocampal gyrus
47	0.967	8	-58	-46	R cerebellar WM
36	0.983	-20	-92	10	L optic radiation
35	0.967	-39	-14	-24	Subcortical WM of the L inferior temporal gyrus
24	0.958	-58	-40	-3	Subcortical WM of the L superior temporal gyrus

Footnote: CAC+: High risk (AA/AG genotypes); CAC-: Low risk (GG genotypes); SZ: Schizophrenia.
[a] Only tracts with clusters at >1% probability are included. White matter labels are provided in accordance with JHU ICBM-DTI-81 White Matter Atlas (Mori et al., 2008) using AtlasQuery in FSL unless marked with "", in which case they were labelled by FC based on MRI Atlas of Human White Matter, 1st Edition by Mori et al., 2005 (Mori et al., 2005) due to retrieval from AtlasQuery returning as 'Unclassified'.*

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Acknowledgements

DP was supported by UK National Institute for Health Research fellowship (NIHR, PDF-2010-03-047), a Marie Curie Career Integration grant (FP7-PEOPLE-2013-CIG-631952) and a Fundação para Ciência e Tecnologia (FCT) Investigator grant (IF/00787/2014**).** EB was supported by a Medical Research Council (MRC) New Investigator Award (G0901310) and the Wellcome Trust (085475/B/08/Z and 085475/Z/08/Z). SS is supported by a European Research Council (ERC) Consolidator award and the NIHR BRC for Mental Health at the South London and Maudsley NHS Foundation Trust.

Authors' contributions

EM carried out the processing and pre-processing of DTI data, conducted the analyses and drafted the manuscript. CC, SS, JW, CM, MP, EK, SK and EB collected the original scan data and some of the genetic data. RM contributed to the design of the project. FC carried out the labelling of the WM and provided guidance in the processing of DTI data. GB provided guidance in DTI data processing and interpretation of findings. DP designed, co-ordinated and supervised the study, collected part of the genetic data, provided guidance in the statistical analysis and joined in drafting the manuscript. All authors read, revised and approved the final manuscript.

Financial Disclosures

GJB received honoraria for teaching from General Electric Healthcare during the course of this research, and acts as a consultant for IXICO. No other author reports biomedical financial interested or declared potential conflicts of interest.